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14. ABSTRACT N-cadherin is a cell surface marker that is overexpressed in hormone refractory prostate cancer and targeting this protein either diagnostically or therapeutically may have clinical utility. We initially observed that the cadherin profiles of certain prostate cancer cell lines and xenografts correlated with their level of invasiveness. In addition, we saw that amongst certain hormone refractory xenograft models, there was a consistent upregulation of N-cadherin when compared to its androgen dependent counterpart. We generated specific monoclonal antibodies against different domains of N-cadherin in an attempt to determine if blockade could decrease invasion and metastasis. For the second year of the grant, there has been an increased focus on the in vitro and in vivo effects of two specific antibodies that were generated.					
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INTRODUCTION

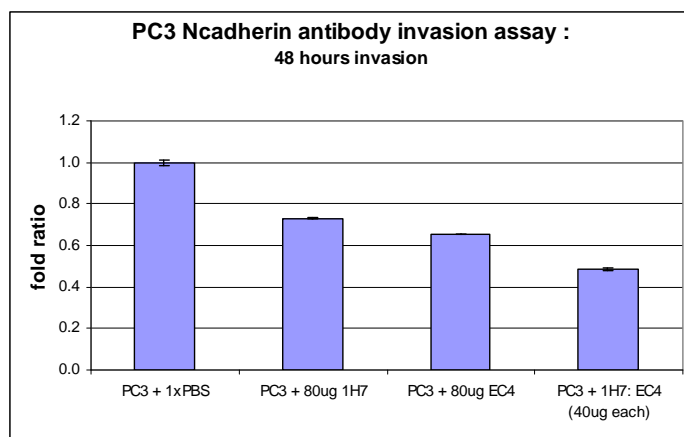
The process known as Epithelial-Mesenchymal Transition (EMT) continues to be implicated as a crucial mediator of carcinogenesis. This phenomenon is manifested by “Cadherin Switching” in which Epithelial (E) Cadherin is lost and Neuronal (N) Cadherin is upregulated. In preliminary studies, we observed that the cadherin profiles of certain prostate cancer cell lines and xenografts correlated with their level of invasiveness. Preliminary evidence suggested that blocking the switch from E-Cadherin to N-Cadherin may block motility and invasiveness of prostate cells in vitro. We sought to validate N-Cadherin as a target for diagnostics or therapeutic purposes in hormone refractory prostate cancer and determine if blockade of this pathway could decrease invasion and metastasis. Previous studies have demonstrated that certain parts of the protein have unique activity and contribute in different ways to the various functions of N-cadherin. As a result, we generated two specific monoclonal antibodies against N-cadherin; one against the first extracellular domain and one against the 4th extracellular domain.

PROGRESS REPORT

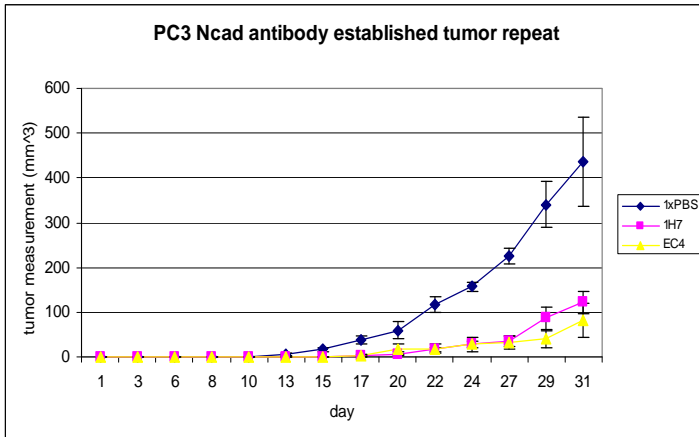
Specific Aim 1. *To validate N-cadherin as target for therapy.*

Subaim1.3: Role of N-cadherin in prostate cancer:

We hypothesized that N-Cadherin may play a role in androgen independent and metastatic progression based on their known roles in other malignancies. In order to test these possibilities in prostate cancer, we generated constructs of N-cadherin and cloned them into CCR lentivirus vectors. Invasiveness of N-cadherin positive cells was assessed by Boyden chamber assays along with neutralizing the N-cadherin positive cells with our N-cadherin generated antibody. PC3 is highly invasive and expresses high N-cadherin levels. Invasion was decreased with antibody treatment.



Below, the left panel shows the in vivo growth curve for PC3 following tumor establishment. There is a clear difference between antibody treated animals versus control. Antibody treatment with 1H7 (directed against the first Extracellular domain) and EC4 (targeting the 4th ECD) was started on day 17, when tumors were palpable. Antibody was given twice weekly for two weeks. Animals were sacrificed when control tumors reached the maximum limits. The right panel shows control tumors being invasive, adherent, and angiogenic. The treated tumors were pale white, non-adherent and noninvasive. Staining of tumors is currently for N-cadherin and other related markers is currently ongoing.



Dissections of PC3 tumors

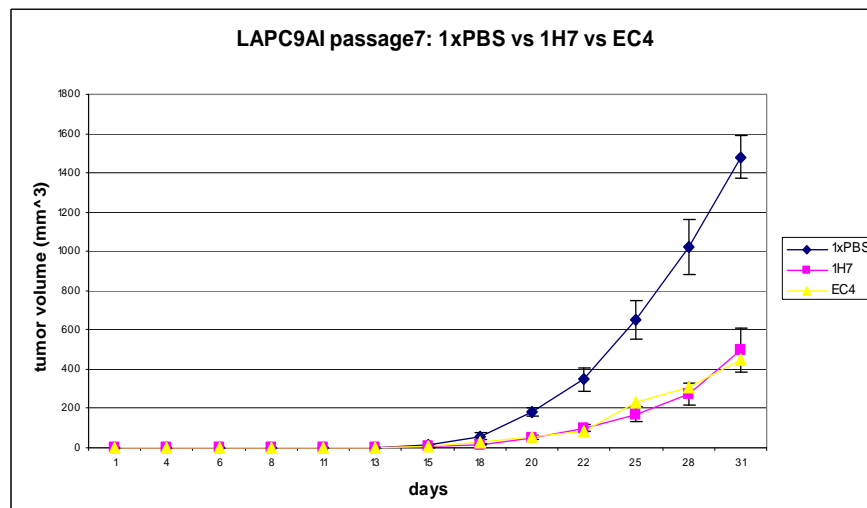


Specific Aim 3. Preclinical testing

Subaim 3.1 Intact fully human antibodies for *in vitro* and *in vivo* pre-clinical testing

To study the involvement of N-cadherin, we looked at the effect of our antibodies against N-cadherin on tumor growth. LAPC9AI cells were injected into castrated mice and treated with N-cadherin antibody when tumors became palpable. There is a clear tumor growth inhibition. Tumors were harvested for histological and molecular analysis.

LAPC9AI passaged into castrated mice

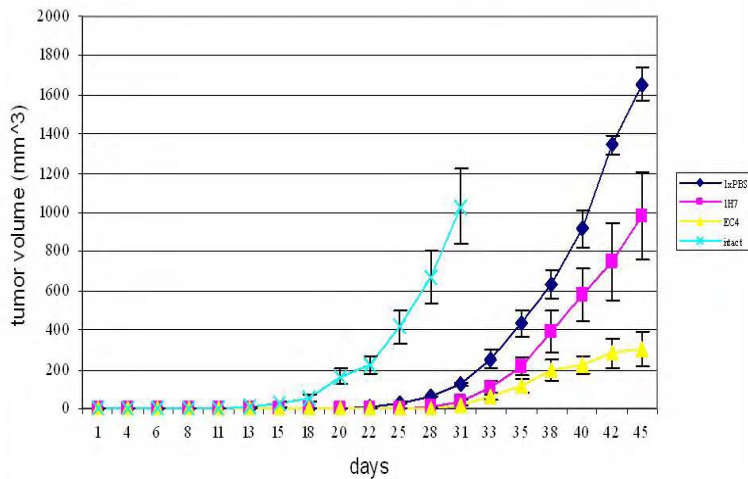


Subaim 3.2 *In vivo* testing of progression to androgen independence

LAPC9AD cells were injected into castrated mice along with an intact control. Antibody treatment began when tumors became palpable. Again, there is a growth inhibition in antibody treated tumors and the

tumors appeared to have similar morphology as the treated tumors described above. Tumors were excised for further analysis. This experiment is currently being repeated to confirm these provocative results.

LAPC9AD to AI transition: 1xPBS vs 1H7 vs EC4



LAPC9AD to AI transition exp using Ncad antibodies

1x PBS



1H7



EC4



KEY RESEARCH ACCOMPLISHMENTS

- Lab generated N-cadherin antibodies appear to have an effect on tumor growth both in vitro and in vivo, especially antibody containing only fourth extracellular domain.
- Gross tumor morphology gives a hint on the mechanism of N-cadherin antibodies as perhaps being related to anti-angiogenic mechanism.

REPORTABLE OUTCOMES

None

CONCLUSION

We have developed and several monoclonal anti-N-cadherin antibodies that have been validated both by immunohistochemistry (with positive and negative controls) as well as with invasion assays. We have demonstrated in several xenograft mouse models that N-cadherin-antibody treatment decreases the size and invasiveness of implanted tumors. Preliminary evidence has pointed to several possible mechanisms of action including inhibition of angiogenesis. Ongoing studies are being performed to determine if inhibition of N-cadherin can delay the development of androgen independence.

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